

What is Claimed is:

1. A method for producing a human-like glycoprotein in a lower eukaryotic host cell comprising the step of expressing in the cell a mannosidase enzymatic activity that is capable of hydrolyzing an oligosaccharide substrate comprising either or both a $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ glycosidic linkage to the extent that at least 10% of the $\text{Man}\alpha 1,3$ and/or $\text{Man}\alpha 1,6$ linkages of the substrate are hydrolyzed *in vivo*.
2. A method for producing a desired N-glycan in a lower eukaryotic host cell comprising the step of expressing in the cell a mannosidase enzymatic activity that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ glycosidic linkage wherein the desired N-glycan is produced within the host cell at a yield of at least 10 mole percent.
3. The method of claim 2, wherein the desired N-glycan produced is selected from the group consisting of $\text{Man}_3\text{GlcNAc}_2$, $\text{GlcNAcMan}_3\text{GlcNAc}_2$ and $\text{Man}_4\text{GlcNAc}_2$.
4. The method of claim 2, wherein the desired N-glycan is characterized as having at least the oligosaccharide branch $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$.
5. The method of claim 1 or 2, wherein the mannosidase enzymatic activity is capable of hydrolyzing *in vivo* both $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ linkages of an oligosaccharide substrate comprising a $\text{Man}\alpha 1,3$ and $\text{Man}\alpha 1,6$ glycosidic linkage.
6. The method of claim 1 or 2, wherein the oligosaccharide substrate is characterized as $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$; $\text{GlcNAc}\beta 1,2$ $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$; $\text{GlcNAc}\beta 1,2$ $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$; $\text{GlcNAc}\beta 1,2$ $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,2$ $\text{Man}\alpha 1,3$ ($\text{Man}\alpha 1,3$ $\text{Man}\alpha 1,6$ $\text{Man}\alpha 1,6$) $\text{Man}\beta 1,4\text{-GlcNAc}$ $\beta 1,4\text{-GlcNAc-Asn}$; $\text{Man}\alpha 1,2$ $\text{Man}\alpha 1,3$

(Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,2 Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn or high mannan.

7. The method of claim 1 or 2, wherein the mannosidase activity is characterized as a Class 2 mannosidase activity.

8. The method of claim 7, wherein the Class 2 mannosidase activity has a substrate specificity for GlcNAc β 1,2 Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; GlcNAc β 1,2 Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; or GlcNAc β 1,2 Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn.

9. The method of claim 7, wherein the Class 2 mannosidase activity is one which is normally found in the Golgi apparatus of a higher eukaryotic host cell.

10. The method of claim 1 or 2, wherein the mannosidase activity is characterized as a Class IIx mannosidase activity.

11. The method of claim 10, wherein the Class IIx mannosidase activity has a substrate specificity for Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; or Man α 1,2 Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn.

12. The method of claim 1 or 2, wherein the mannosidase activity is characterized as a Class III mannosidase activity.

13. The method of claim 12, wherein the Class III mannosidase activity has a substrate specificity for (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; or high mannans.

14. The method of claim 1 or 2, wherein the mannosidase activity is overexpressed.

15. The method of claim 1 or 2, wherein the mannosidase is further capable of hydrolyzing a Man α 1,2 linkage.

16. The method of claim 1 or 2, wherein the mannosidase activity has a pH optimum of from about 5.0 to about 8.0.

17. The method of claim 1 or 2, wherein the mannosidase is further capable of hydrolyzing a Man α 1,2 linkage.

18. The method of claim 1 or 2, wherein the mannosidase activity is localized within the secretory pathway of the host cell.

19. The method of claim 1 or 2, wherein the mannosidase activity is expressed from a polypeptide localized within at least one of the ER, Golgi apparatus or the trans golgi network of the host cell.

20. The method of claim 1 or 2, wherein the mannosidase activity is expressed from a nucleic acid encoding a polypeptide comprising a mannosidase catalytic domain fused to a cellular targeting signal peptide.

21. The method of claim 20, wherein the mannosidase activity is expressed from a nucleic acid comprising sequences that encode a mannosidase catalytic domain native to the host cell

22. The method of claim 20, wherein the mannosidase activity is expressed from a nucleic acid comprising sequences that encode a mannosidase catalytic domain heterologous to the host cell.

23. The method of claim 1 or 2, wherein the mannosidase enzymatic activity is selected from the group consisting of *Arabidopsis thaliana* Mannosidase II, *C. elegans* Mannosidase II, *Ciona intestinalis* mannosidase II, *Drosophila* mannosidase II, Human mannosidase II, Mouse mannosidase II, Rat mannosidase II, Human mannosidase IIx, Insect cell mannosidase III, Human lysosomal mannosidase II and Human cytoplasmic mannosidase II.

24. The method of claim 1 or 2, wherein the polypeptide is expressed from a nucleic acid comprising sequences that encode a target peptide native to the host cell.

25. The method of claim 1 or 2, wherein the polypeptide is expressed from a nucleic acid comprising sequences that encode a target peptide heterologous to the mannosidase catalytic domain.

26. The method of claim 1 or 2, further comprising the step of isolating the glycoprotein from the host cell.

27. The method of claim 1 or 2, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, *Fusarium gramineum*, *Fusarium venenatum* and *Neurospora crassa*.

28. The method of claim 27, wherein the host cell is *Pichia pastoris*.

29. The method of claim 1 or 2, wherein the glycoprotein is a therapeutic protein.

30. The method of claim 29, wherein the therapeutic protein is selected from the group consisting of erythropoietin, cytokines, coagulation factors, soluble IgE receptor α -chain, IgG, IgG fragments, IgM, interleukins, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α -1-antitrypsin and α - fetoprotein.

31. A nucleic acid library comprising at least two different genetic constructs, wherein at least one genetic construct comprises a nucleic acid fragment encoding a mannosidase class 2, IIx or III catalytic domain ligated in-frame with a nucleic acid fragment encoding a cellular targeting signal peptide which it is not normally associated with.

32. The library of claim 31, wherein the mannosidase catalytic domain is selected from the group consisting of *Arabidopsis thaliana* Mannosidase II, *C. elegans* Mannosidase II, *Ciona intestinalis* mannosidase II, *Drosophila* mannosidase II, Human mannosidase II, Mouse mannosidase II, Rat mannosidase II, Human mannosidase IIX, Insect cell mannosidase III, Human lysosomal mannosidase II and Human cytoplasmic mannosidase II.

33. The library of claim 31, wherein the nucleic acid fragment encoding a cellular targeting peptide is selected from the group consisting of: *Saccharomyces* GLS1, *Saccharomyces* MNS1, *Saccharomyces* SEC12, *Pichia* SEC, *Pichia* OCH1, *Saccharomyces* MNN9, *Saccharomyces* VAN1, *Saccharomyces* ANP1, *Saccharomyces* HOC1, *Saccharomyces* MNN10, *Saccharomyces* MNN11, *Saccharomyces* MNT1, *Pichia* D2, *Pichia* D9, *Pichia* J3, *Saccharomyces* KTR1, *Saccharomyces* KTR2, *Kluyveromyces* GnT1, *Saccharomyces* MNN2, *Saccharomyces* MNN5, *Saccharomyces* YUR1, *Saccharomyces* MNN1, and *Saccharomyces* MNN6.

34. A vector comprising a fusion construct derived from a library of any one of claims 31-33 operably linked to an expression control sequence, wherein said cellular targeting signal peptide is targeted to at least one of the ER, Golgi or trans-Golgi network.

35. The vector of claim 34, wherein the expression control sequence is inducible or constitutive.

36. The vector of claim 34 which, upon expression in a host cell, encodes a mannosidase activity involved in producing GlcNAcMan₃GlcNAc₂ Man₃GlcNAc₂ or Man₄GlcNAc₂ *in vivo*.

37. A host cell comprising at least one vector of claim 36.

38. A host cell comprising at least one vector selected from the group of vectors designated pKD53, pKD1, pKD5, pKD6 and pKD16.

39. A chimeric polypeptide comprising a mannosidase catalytic domain fused in-frame to a targeting signal peptide and, upon expression in a lower eukaryotic host cell, capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Man α 1,3 and Man α 1,6 glycosidic linkage to the extent that at least 10% of the Man α 1,3 and/or Man α 1,6 linkages of the substrate are hydrolyzed *in vivo*.

40. A chimeric polypeptide comprising a mannosidase catalytic domain fused in-frame to a targeting signal peptide and, upon expression in a lower eukaryotic host cell, capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising a Man α 1,3, Man α 1,6, or Man α 1,2 glycosidic linkage to the extent that a detectable moiety of the Man α 1,3, Man α 1,6 or Man α 1,2 linkage of the substrate is hydrolyzed *in vivo*.

41. A nucleic acid encoding a chimeric polypeptide of claim 39.

42. A host cell comprising a chimeric polypeptide of claim 39.

43. A host cell comprising a nucleic acid of claim 41.

44. A glycoprotein produced in a host cell of claim 42 or claim 43.

45. An N-glycan produced in a host cell of claim 42 or claim 43.

46. The N-glycan of claim 45, wherein the N-glycan is characterized as uniform.

47. A glycoprotein produced by the method of claim 1 or claim 2.

48. An N-glycan produced by the method of claim 1 or claim 2.

49. The N-glycan of claim 48, wherein the N-glycan is characterized as uniform.

50. An isolated polynucleotide comprising or consisting of a nucleic acid sequence selected from the group consisting of:

(a) SEQ ID NO: 92 (*C.elegans* **FROM FIG. 23**);

(b) at least about 90% similar to the amino acid residues of the donor

nucleotide binding site of SEQ ID NO: 92;

(c) a nucleic acid sequence at least 92%, at least 95%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 93;

(d) a nucleic acid sequence that encodes a conserved polypeptide having the amino acid sequence of SEQ ID NO: 92;

(e) a nucleic acid sequence that encodes a polypeptide at least 78%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 92;

(f) a nucleic acid sequence that hybridizes under stringent conditions to SEQ ID NO: 92; and

(g) a nucleic acid sequence comprising a fragment of any one of (a) – (f) that is at least 60 contiguous nucleotides in length.

51. An isolated polynucleotide comprising or consisting of a nucleic acid sequence selected from the group consisting of:

(a) SEQ ID NO: 93(rat **FROM FIG. 23**);

(b) at least about 95% similar to the amino acid residues of the donor nucleotide binding site of SEQ ID NO: 93;

(c) a nucleic acid sequence at least 95%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 93;

(d) a nucleic acid sequence that encodes a conserved polypeptide having the amino acid sequence of SEQ ID NO: 93;

(e) a nucleic acid sequence that encodes a polypeptide at least 97%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 93;

(f) a nucleic acid sequence that hybridizes under stringent conditions to SEQ ID NO: 93; and

(g) a nucleic acid sequence comprising a fragment of any one of (a) – (f) that is at least 60 contiguous nucleotides in length.

52. An isolated polynucleotide comprising or consisting of a nucleic acid sequence selected from the group consisting of:

(a) SEQ ID NO: 94(Ciona **FROM FIG. 23**);

- (b) at least about 90% similar to the amino acid residues of the donor nucleotide binding site of SEQ ID NO: 94;
- (c) a nucleic acid sequence at least 92%, at least 95%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 94;
- (d) a nucleic acid sequence that encodes a conserved polypeptide having the amino acid sequence of SEQ ID NO: 94;
- (e) a nucleic acid sequence that encodes a polypeptide at least 73%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 94;
- (f) a nucleic acid sequence that hybridizes under stringent conditions to SEQ ID NO: 94; and
- (g) a nucleic acid sequence comprising a fragment of any one of (a) – (f) that is at least 60 contiguous nucleotides in length.

53. An isolated polynucleotide comprising or consisting of a nucleic acid sequence selected from the group consisting of:

- (a) SEQ ID NO: 95(Arabidopsis **FROM FIG. 23**);
- (b) at least about 95% similar to the amino acid residues of the donor nucleotide binding site of SEQ ID NO: 95;
- (c) a nucleic acid sequence at least 96%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 95;
- (d) a nucleic acid sequence that encodes a conserved polypeptide having the amino acid sequence of SEQ ID NO: 95;
- (e) a nucleic acid sequence that encodes a polypeptide at least 95%, at least 98%, at least 99% or at least 99.9% identical to SEQ ID NO: 95;
- (f) a nucleic acid sequence that hybridizes under stringent conditions to SEQ ID NO: 95; and
- (g) a nucleic acid sequence comprising a fragment of any one of (a) – (f) that is at least 60 contiguous nucleotides in length.

54. A modified polynucleotide comprising or consisting of a nucleic acid sequence selected from the group consisting of the conserved regions SEQ ID NO: 5 –

SEQ ID NO: 15 wherein the encoded polypeptide is involved in hydrolyzing a Man α 1,3 and/or a Man α 1,6 glycosidic linkage of an oligosaccharide.

55. A modified polynucleotide comprising or consisting of a nucleic acid sequence selected from the group consisting of the conserved regions of SEQ ID NO: 49 – SEQ ID NO: 59 wherein the encoded polypeptide is involved in hydrolyzing a Man α 1,3 and/or a Man α 1,6 glycosidic linkage of an oligosaccharide.

56. A vector selected from the group consisting of pKD53, pKD1, pKD5, pKD6 and pKD16.